

**3573-Pos Board B434****Tissue Geometry Regulates Collective Cell Motility**

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Collective cell migration plays an important role in embryonic development, wound repair and cancer invasion. Certain cell types have an intrinsic ability to organize themselves and move collectively when they are confined within monolayers. Here, we explored the role of tissue geometry on the collective motility of epithelial cells. In particular, we used microlithography and time-lapse imaging to ask whether tissue geometry affects multicellular polarity and supracellular organization that are necessary for collective cell motion. We found that epithelial cells within monolayers tended to rotate as a group, and that increasing the size of the tissue increased the collectiveness of group rotation. The shape and boundary conditions of the tissue organized the motion of the cells by altering group rotation and coherence. The motility parameters of individual cells, including speed and persistence, were also affected by tissue geometry. Our results suggest that the overall architecture of the tissue in which cells reside instructs their movements with respect to each other within a collective. Accurate recapitulation of *in vivo* tissue structure will benefit future studies of processes which involve collective cell migration.

**3574-Pos Board B435****Fibronectin Matrix Assembly Regulates Shear Stress-Induced Structural Remodeling and Motility**

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Complex spatial profiles of shear stress have been implicated in focal development of atherosclerotic lesions by mechanisms involving force transmission through endothelial cell (EC) focal adhesions to cytoskeleton. Fibronectin is a primary component of the provisional extracellular matrix (ECM) deposited in atherosclerotic lesions. Integrin-mediated mechanosignaling pathways in ECs are required for both adaptation to shear stress and fibronectin fibrillogenesis, but how fibronectin assembly state modulates EC responses to shear stress remains unknown. To investigate this question, focal adhesion displacement, cytoskeletal reorganization, and migration were measured before and after a step increase from 0 to 12 dyn/cm<sup>2</sup> steady unidirectional shear stress acting on ECs interacting with either assembled, fibrillar fibronectin matrix or unassembled, fragmented fibronectin on glass. Shear stress onset induced the arrest of focal adhesion displacement in ECs on unassembled fibronectin but not in cells on assembled fibronectin. In subconfluent layers, ECs on unassembled fibronectin migrated downstream after shear stress onset, but cells on assembled fibronectin migrated in random directions associated with local fibril orientations. In confluent monolayers, ECs interacting with unassembled fibronectin aligned in the flow direction faster than in monolayers on fibrillar fibronectin matrix, suggesting that fibronectin assembly regulated shear stress-induced cytoskeletal remodeling. Since cytoskeletal remodeling and focal adhesion displacement reflect early mechanosensing events after the onset of shear stress, these data suggest that fibronectin assembly regulates mechanosensitivity at the cell-matrix interface, which leads to shear stress-induced adaptation of cell motility and alignment.

**3575-Pos Board B436****Human Mesenchymal Stem Cells Migration on Matrices with Distinct Elasticity Gradient Magnitudes**

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Adult mesenchymal stem cells (MSCs) respond to extracellular niche elasticity, which varies dramatically between tissues that MSCs inhabit. Similarly, as MSCs egress from bone marrow and bone to tissues, they may encounter stiffness gradients brought on either by pathological conditions, e.g. myocardial infarction  $\sim 8.7 \pm 1.5$  kPa/mm, or through normal tissue variation, e.g. muscle  $\sim 0.6 \pm 0.9$  kPa/mm. We have recently shown that MSCs can undergo directed migration in response to shallow, physiological ( $\sim 1$  kPa/mm) stiffness gradients before differentiating, suggesting the importance of spatial changes in stiffness. Such gradients, however, contain aphysical ranges, e.g. 1-15 kPa, and more refined gradients of both range and gradient strength that mimic tissue interfaces are needed to better understand how mechanical cues dictate MSC migration versus differentiation. Using a polydimethylsiloxane micro-channel mixer, we generated a polymer solution with a one-dimensional crosslinker concentration of constant monomer and photoinitiator but varying

crosslinker. Photopolymerizing the solution inside the device yields a 3mm wide hydrogel with varying mechanical properties. Stiffness gradients of varying strength (1-30 kPa/mm) and range (0.1-100 kPa) are achieved by varying the relative concentration of crosslinker from the input solutions. MSCs responded to stiffness gradients with a physiological range, e.g. mimicking the myotendinous junction, but of varying strength, i.e. 1-30 kPa/mm. However, migration velocities of MSCs on gels of varying gradient strength were similar. Cell morphology was stiffness dependent with cells exhibiting increased spread areas on the stiffer regions, suggesting that the previously observed correlation between substrate mechanics, cell motility, and morphology exists over a physiological stiffness range but is independent of gradient strength. Efforts to define optimal myogenic stiffness and studies with C2C12 muscle cells are ongoing. These findings imply that MSCs *in vivo* may contribute better to repairs in stiffer regions of tissues where they may preferentially accumulate.

**3576-Pos Board B437****Dynamic Surface Topography Influences Cell Function**

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Micro- and nano-scale changes in surface topography can modulate mesenchymal stem cell (MSC) differentiation; rough surfaces have been shown to induce osteogenesis to varying degrees depending on the scale and nature of the topographical features. While responses to static surfaces are novel, topography *in vivo* is constantly being remodeled by cells within the niche. To better understand how mesenchymal stem cells respond to changes in topography over time, we developed a soft polyacrylamide hydrogel with magnetic nickel microwires randomly oriented in the surface of the material. Varying the magnetic field around the microwires can reversibly induce their alignment with the direction of the field, causing the smooth hydrogel surface to develop small wrinkles. By varying the density of wires in the hydrogel, surface roughness changes,  $\Delta R_{RMS}$ , can range from 0.05 to 0.62  $\mu$ m as measured by traction force microscopy; this range encompasses roughness values for static surfaces. Time-dependent topographical changes are achieved by oscillating the field around the microwires, and ongoing efforts using step function and cyclic changes in topography indicate that both smooth muscle cells and MSCs change their behavior in response to dynamic surface topography, e.g. distinct changes in cytoskeletal structure as well as lineage marker expression is expected. Being able to dynamically study how cells respond to changes in topography will improve our current understanding of topography-driven effects on stem cell differentiation.

**3577-Pos Board B438****Nanotopography Driven Mesenchymal Stem Cell Differentiation and Proliferation**

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Plastic-adherent, fibroblast-like populations of bone marrow cells, termed multipotent mesenchymal stromal/stem cells (MSCs), constitute a tissue specific stem cell population that is responsible for tissue homeostasis and repair of bone, cartilage and adipose tissue. Previous work with MSCs had shown that they are capable of differentiation into specific lineages in response to the physical properties of the extracellular matrix such as matrix elasticity and cell geometry. Remarkably MSCs have been demonstrated to differentiate into bone-forming osteoblast cells by modifying the substrate nanotopography. In this work, the role of nanotopography on the long-term response of MSCs is explored using biodegradable polycaprolactone (PCL) nanopillar and nanofiber surfaces seeded with rat MSCs and cultured in normal growth media for four weeks. We found that after four weeks in culture under normal expansion media conditions, MSCs cultured on nanofibers exhibit better adherence, significantly increased proliferation, and maintain increasingly dense fibroblast-like morphologies. In contrast, MSCs seeded on nanopillar surfaces display lowered adherence, reduced proliferation, and adopt highly elongated cellular morphologies. Cell shape and area quantification reveals that MSCs cultured on smooth substrates adopt uniformly spread morphologies covering large surface areas, while MSCs cultured on nanopillars have significantly larger length-to-width ratios and significantly reduced coverage areas. Immunofluorescent staining of MSCs on PCL nanopillars reveals the presence of two bone marker proteins, osteopontin and osteocalcin, providing evidence for differentiation into osteoblast-like cells. Unlike the nanopillar topography, MSCs cultured on nanofiber and smooth PCL surfaces did not appear to

undergo surface induced osteogenesis. We put forward a mechanotransductive model to interpret these and other's findings.

#### 3578-Pos Board B439

##### **Mesenchymal Stem Cells Duro- and Hapto- Taxis Alters Cell State**

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Anchorage dependent cells can sense and respond to extracellular matrix (ECM) stiffness, but stiffness gradients are often found in vivo via normal tissue variation or pathological conditions, such as the post-infarct myocardial scar which is several folds stiffer than healthy tissue. We have previously shown that mesenchymal stem cell (MSC) differentiation as well as migration is regulated by substrate stiffness in 2D in vitro; it is important to determine if this also occurs in 3D as it could explain MSC migration and calcification in infarct scars in vivo. 3 mg/ml collagen hydrogels were crosslinked with 1, 5, 10, and 20 mM genipin, a natural nontoxic collagen crosslinker, for 2, 4, 12, 24, and 48 hours to yield physiological stiffnesses ranging from 0.9 to 6.4 kPa. MSC behavior on these matrices mimicked previous reports. 3D gradient hydrogels with encapsulated MSCs, fabricated using microfluidics to diffuse genipin through a cell-collagen network, should result in a spatial gradient of crosslinking across the gel. MSCs proliferation, migration, morphology, and differentiation in the presence of this 3D gradient will be compared with 2D gradients in which MSCs were observed to first migrate and then differentiate. MSC durotaxis during wound healing in vivo may be additionally accompanied by haptotaxis, migration due to a matrix ligand gradient, as a result of localized matrix secretion by fibroblasts at the site of regeneration. 2D haptotactic hydrogels with gradients of collagen and fibronectin were also made and can be overlaid onto stiffness gradient hydrogels in order to investigate the effect of corresponding and opposing gradients on MSC behavior. These data show that MSC migration and subsequent differentiation can be regulated by a variety of ECM stimuli in addition to growth factor-mediated pathways.

#### 3579-Pos Board B440

##### **Natural Killer Cell-Mediated Tumor Surveillance: Correlation Between Killing Efficiency, Transient Migration Behavior and Morphology**

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We recently developed a simple method for automatic characterization of transient migration behavior of natural killer (NK) cells imaged by fluorescence time-lapse microscopy [1]. For imaging we used a microchip-based assay where small numbers of NK and tumor target cells were confined inside micro-wells. Single-cell tracking revealed differences between NK cells as well as transient changes in migration behavior for individual NK cells. Frequently, NK cells were found to have periods of high motility, interrupted by periods of slow migration or complete stops. Such transient migration arrest periods (TMAPs) often coincided with formation of immune synapses with target cells, but NK cells were also observed to stop spontaneous and prior to cell division or death. To objectively characterize NK cell migration, we used a sliding window approach to evaluate local migration coefficients and mean squared displacement curvature in individual cell trajectories. Three modes of migration were distinguished: TMAPs, directed migration and random movement. Here, we correlated transient migration behavior with NK cell morphology. In general, NK cells were more circular during immune synapse formation and in TMAPs compared to when in directed migration or random movement. On the other hand, motile NK cells had bigger areas and perimeters compared to NK cells engaged in immune synapses or in TMAPs. The method presented can quickly and quantitatively assess migration and morphological properties of different cell populations. Current analysis is focused on determining whether particularly cytotoxic NK cells (serial killers) display distinct properties from other subpopulations of NK cells.

1. Khorshidi, M.A., et al., *Analysis of transient migration behavior of natural killer cells imaged in situ and in vitro*. Integr Biol (Camb), 2011. 3(7): p. 770-8.

#### 3580-Pos Board B441

##### **Cancer Migration in 3-D Environment**

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Cell migration plays an important role in various biological processes ranging from tissue repair to cancer metastasis. Most of the studies reported so

far have investigated cell migration in a 2-D environment. However, recent years have witnessed a surge of interest in understanding migration of cells within 3-D environment recapitulating various attributes of the native environment. To this end, we have developed an approach to study the migratory transition from 2-D to 3-D on Matrigel as a model system to mimic the processes, including matrix degradation, occurring in 3-D migration. Using this system, we characterize the migration capabilities of non-metastatic (MCF-7) and metastatic (MDA-MB-231) breast cancer cells by analyzing (i) the penetration depth into the Matrigel required for plastic deformation of the matrix, (ii) the magnitude and distribution of traction forces generated before the elastic limit of the material. Furthermore, we examine whether the cellular motility between non-metastatic and metastatic cells converge to a similar behavior when the matrix metalloproteinase activity are altered at various levels. The degree of convergence will be determined by again analyzing the required depth for plastic deformation and the magnitude and distribution of traction forces generated before the elastic limit.

#### 3581-Pos Board B442

##### **Differential Effects of Serum Heat Treatment on Chemotaxis and Phagocytosis by Human Neutrophils**

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The ability of certain white blood cells to actively change their shape is not only a cornerstone of a host's innate immune defense, but also provides a highly instructive, cross-disciplinary window into eukaryotic cell motility. Recent findings have indicated that human neutrophils rely at least partially on similar biophysical mechanisms during chemotaxis and phagocytosis. To further elucidate similarities and differences between these two prominent examples of immune-cell motility, we here examine the effects of heat treatment of serum components on neutrophil interactions with antibody-coated beads and zymosan particles. Because heat treatment can inhibit some or all viral activity in the serum, it is a common method to protect laboratory personnel, and also has many other applications. Here, we incubate neutrophils with the two target types in buffers containing serum that had been treated at a range of different temperatures. To discriminate between phagocytic and chemotactic behavior, we quantify the cell response by combining flow cytometry bulk assays with single-cell/single-target experiments based on dual-micropipette manipulation. We find that heat treatment of serum at 52°C or higher significantly depressed phagocytosis of opsonized zymosan in our bulk assays for all donors tested. On the other hand, single-cell experiments demonstrated complete inhibition of chemotactic activity already at 48°C, even though the neutrophils still engulfed target particles that were brought into physical contact with the cells using micropipettes. This implies that, although chemotactic stimulation of neutrophils is not required for phagocytosis, it can significantly enhance the phagocytic response. Our results also demonstrate that by fine-tuning heat treatment of serum, one can selectively study chemotaxis or phagocytosis under otherwise identical conditions.

#### 3582-Pos Board B443

##### **Tangential Tether Extraction and Spontaneous Tether Retraction of Human Neutrophils**

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Membrane tethers are extracted when neutrophils roll on the endothelium to initiate their transendothelial migration. Tether extraction from both neutrophils and endothelial cells stabilizes neutrophil rolling, so it has been studied extensively and the force-velocity relationship for tether extraction is of great interest. Limited by the techniques in previous studies, this relationship has been only obtained from tethers perpendicular to the cell surface. Here, with the micro-cantilever technique where latex beads affixed on silicon cantilevers were used as the force transducer, we extracted tethers either perpendicular or tangential to the neutrophil surface. We found that the force-velocity relationship was not sensitive to tether pulling direction. Little movement of the tether-cell junction was observed during tangential tether extraction and no coalescence was observed during multiple tether extraction. Following adhesion rupture, spontaneous tether retraction was visualized by membrane staining, which revealed two phases: one was fast and exponential, whereas the other was slow and linear. Our mechanistic modeling findings showed some similar tether behaviors. Therefore, these results illustrate for the first time how neutrophil tethers shorten upon instantaneous force removal and illustrate further how membrane tethers contribute to neutrophil rolling stability during the inflammatory response.